Endogenous MCM7 MicroRNA Cluster as a Novel Platform to Multiplex Small Interfering and Nucleolar RNAs for Combinational HIV-1 Gene Therapy.

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Public Summary:
The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus type 1 (HIV-1). Although the current standard highly active antiretroviral therapy is efficient in keep viral replication in check, it is not curative. Gene therapy approaches are attractive alternatives where the most vulnerable immune cells are modified with antiviral small RNAs with the goal to engineer an HIV-resistant immune system. In this current study, we utilized an endogenous microRNA (miRNA) cluster as a platform to express multiple anti-HIV small RNAs. This multiplex approach reduces the likelihood of viral resistance and enhances the synergistic potential between inhibitory agents with different mechanism of action and targets, with the overall goal of achieving long-term protection against HIV. One class of such RNA agents is small interfering RNAs (siRNAs) that mediate viral message degradation with perfect base pairing to the target. The other class of anti-HIV RNAs is self-catalytic ribozymes that recognizes their target by base pairing following by enzymatic cutting of the target. Finally, anti-HIV RNA decoys that aim to titrate out early essential viral replication proteins Tat and Rev are constructed. We demonstrated persistent expression of each anti-HIV RNAs in CEM T cell lines that stably integrate the transgene. Moreover, combinations with small RNAs capable of turning over multiple target substrates, such as siRNAs and ribozymes, showed greater inhibition to HIV replication in a one-month challenge assay. Interestingly, cells with optimal level of small RNA expression to effectively inhibit viral replication were enriched under the selective pressure of HIV, demonstrating the importance of balancing expression and functionality for successful gene therapy applications.

Scientific Abstract:
Abstract Combinational therapy with small RNA inhibitory agents against multiple viral targets allows efficient inhibition of viral production by controlling gene expression at critical time points. Here we explore combinations of different classes of therapeutic anti-HIV-1 RNAs expressed from within the context of an intronic MCM7 (minichromosome maintenance complex component-7) platform that naturally harbors 3 microRNAs (miRNAs). We replaced the endogenous miRNAs with anti-HIV small RNAs, including small interfering RNAs (siRNAs) targeting HIV-1 tat and rev messages that function to induce post-transcriptional gene silencing by the RNA interference pathway, a nucleolar-localizing RNA ribozyme that targets the conserved U5 region of HIV-1 transcripts for degradation, and finally nucleolar trans-activation response (TAR) and Rev-binding element (RBE) RNA decoys designed to sequester HIV-1 Tat and Rev proteins inside the nucleolus. We demonstrate the versatility of the MCM7 platform in expressing and efficiently processing the siRNAs as miRNA mimics along with nucleolar small RNAs. Furthermore, three of the combinatorial constructs tested potently suppressed viral replication during a 1-month HIV challenge, with greater than 5-log inhibition compared with untransduced, HIV-1-infected CEM T lymphocytes. One of the most effective constructs contains an anti-HIV siRNA combined with a nucleolar-localizing U5 ribozyme and TAR decoy. This represents the first efficacious example of combining Drosha-processed siRNAs with small nucleolar ribonucleoprotein (snoRNP)-processed nucleolar RNA chimeras from a single intron platform for effective inhibition of viral replication. Moreover, we demonstrated enrichment/selection for cells expressing levels of the antiviral RNAs that provide optimal inhibition under the selective pressure of HIV. The combinations of si/snoRNAs represent a new paradigm for combinatorial RNA-based gene therapy applications.